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     (FILE 'HOME' ENTERED AT 08:26:59 ON 07 DEC 2004)

     FILE 'MEDLINE' ENTERED AT 08:27:13 ON 07 DEC 2004
L1       154242 S INDICATOR OR REPORTER GENE
L2          103 S L1 AND SCREEN AND LIBRARY
L3          103 DUP REM L2 (0 DUPLICATES REMOVED)
L4           43 S L2 AND PY <2000
L5            0 S L4 AND GPCR
L6            0 S L2 AND GPCR
L7           33 S L1 AND GPCR
L8            1 S L7 AND LIBRARY
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=> d l4 1-10 ti au py so abs

L4 ANSWER 1 OF 43 MEDLINE on STN
TI Facilitating functional analysis of the Saccharomyces cerevisiae genome using an EGFP-based promoter **library** and flow cytometry.
AU Bell P J; Davies I W; Attfield P V
PY 1999
SO Yeast (Chichester, England), **(1999 Dec)** 15 (16) 1747-59.
Journal code: 8607637. ISSN: 0749-503X.
AB A promoter **library** was generated to facilitate identification of differentially regulated promoters in Saccharomyces cerevisiae. The **library** was constructed in a vector containing two **reporter genes** (EGFP and lacZ) divergently arranged about a unique cloning site. Approximately 2x10(5) clones were obtained and a flow cytometer was used to **screen** the **library** for copper-induced EGFP expression. A DNA fragment conferring copper-inducible expression of EGFP was rapidly identified. This DNA fragment, which contained several motifs associated with copper and oxidative stress homeostasis, lies upstream of two 'orphan' genes of unknown function. Further studies comparing expression from episomal vs. integrative vectors showed that construction of a similar **library** using an integrative vector would further enhance rapid identification of genes that are differentially regulated in S. cerevisiae. The ability to identify regulated promoters rapidly should facilitate the functional analysis of the yeast genome by identifying genes induced by specific physiological conditions.

L4 ANSWER 2 OF 43 MEDLINE on STN
TI Expression of cre recombinase as a reporter of signal transduction in mammalian cells.
AU Mattheakis L C; Olivan S E; Dias J M; Northrop J P
PY 1999
SO Chemistry & biology, **(1999 Nov)** 6 (11) 835-44.
Journal code: 9500160. ISSN: 1074-5521.
AB BACKGROUND: Cell-based reporter assays, which rely on a **reporter gene** under the control of a regulated promoter, are widely used to **screen** chemical **libraries** for novel receptor ligands. Here, we describe a reporter system that is based on ligand-induced DNA recombination to express the **reporter gene**. This system converts a transient activation of a signal transduction pathway into an amplified, constitutive and heritable expression of the **reporter gene**. RESULTS: We constructed gene fusions of Cre recombinase and mammalian promoters regulated by calcium, nuclear receptors or cyclic AMP. Reporter systems, comprising a Cre gene fusion and a loxP/**reporter gene**, were used to study the kinetics and dose responses to compounds that activate or inhibit the corresponding signal transduction pathway. We compared these reporters with conventional reporter systems in which the **reporter gene** is under the direct control of the responsive promoter. **Reporter gene** expression of the Cre reporters was greater than that of conventional reporters and could be measured more than a week after adding the stimulus. For all pathways studied here, the dose responses of the Cre reporters are nearly identical to those of conventional reporter systems. CONCLUSIONS: We have shown that Cre recombinase can be regulated by a variety of signal transduction pathways. It should therefore be possible to use receptor ligands to induce phenotypic conversion of mammalian cells for use in a variety of applications. One such application is high-throughput screening, and we developed loxP/luciferase **reporter genes** that provide an amplified and sustained luminescent response.

L4 ANSWER 3 OF 43 MEDLINE on STN
TI In vivo screening of haloalkane dehalogenase mutants.
AU Chang C H; Schindler J F; Unkefer C J; Vanderberg L A; Brainard J R; Terwilliger T C
PY 1999
SO Bioorganic & medicinal chemistry, **(1999 Oct)** 7 (10) 2175-81.
Journal code: 9413298. ISSN: 0968-0896.
AB Haloalkane dehalogenase (Dh1A) from Xanthobacter autotrophicus GJ10 catalyzes the dehalogenation of short chain primary alkyl halides. Due to the high Km and low turnover, wild type Dh1A is not optimal for applications in bioremediation. We have developed an in vivo **screen**, based on a colorimetric pH **indicator**, to identify Dh1A mutant with improved catalytic activity. After screening 50,000 colonies, we identified a Dh1A mutant with a lower pH optimum. Sequence analysis of the mutant revealed a single substitution, alanine 149 to threonine, which is located close to the active site of Dh1A. Replacement of alanine 149 via site-directed mutagenesis with threonine, serine or cysteine retained the mutant phenotype. Other substitutions at position 149 show little or no activity.

L4 ANSWER 4 OF 43 MEDLINE on STN
TI Staphylococcus aureus nuclease is a useful secretion reporter for mycobacteria.
AU Downing K J; McAdam R A; Mizrahi V
PY 1999
SO Gene, **(1999 Nov 1)** 239 (2) 293-9.
Journal code: 7706761. ISSN: 0378-1119.
AB A secretion reporter system based on Staphylococcus aureus nuclease (nuc) was developed for use in mycobacteria. Fusion of secretion signals to the reporter cloned in a shuttle vector, pBPnuc1, resulted in halo formation around colonies of Mycobacterium smegmatis and Mycobacterium tuberculosis grown on DNase agar plates containing Methyl Green **indicator** dye. This in-situ detection system was used to identify secreted proteins by screening a pBPnuc1::H37Rv nuc gene fusion **library** in M. smegmatis. The clones identified in this **screen** all formed colony halos when present in M. tuberculosis grown on **indicator** media. The proteins corresponded to DesA2, a stearoyl-acyl carrier protein desaturase, PepA, a putative serine protease and the Apa antigen, which is the ATP-binding subunit of an ABC transport system. Of these proteins, only PepA and Apa contained recognizable leader peptides.

L4 ANSWER 5 OF 43 MEDLINE on STN
TI Homogeneous pharmacologic and cell-based **screens** provide diverse strategies in drug discovery: somatostatin antagonists as a case study.
AU Zysk J R; Baumbach W R
PY 1998
SO Combinatorial chemistry & high throughput screening, **(1998 Dec)** 1 (4) 171-83. Ref: 59
Journal code: 9810948. ISSN: 1386-2073.
AB The development of high throughput, homogeneous pharmacologic and functional assays and their implementation in screening combinatorial **libraries** has increased the pace of stochastic drug discovery in recent years. New, noninvasive approaches involving radiometric proximity assays, an array of fluorescence-based technologies, and **reporter gene** constructs in mammalian and nonmammalian systems are providing more options for the selection of specific therapeutic targets. The increasing sophistication of homogeneous assay designs has also served as a springboard to better lead validation in drug discovery initiatives. This review examines these approaches in the context of new drug discovery strategies which combine a growing repertoire of high throughput screening techniques. The utility and importance of cell-based assays vis-a-vis pharmacologic (cell-free) assays is considered with specific reference

given to yeast-based functional **screens** and homogeneous binding methodologies used to search for somatostatin antagonists and other potential growth hormone secretagogues. Also considered is the custom tailoring of specific chemical **libraries** which provide yet another level of target selectivity. The advantages and shortcomings of these various technologies are discussed in light of emerging trends toward higher throughput and nanoscale formats which are pushing the limits of measurable response at the cellular and molecular level.

L4 ANSWER 6 OF 43 MEDLINE on STN
TI Differential sensitivity to 5-fluoro-orotic acid as a **screen** for bait RNA-independent false positives in a yeast three-hybrid system.
AU Park Y W; Tan S L; Katze M G
PY 1999
SO BioTechniques, **(1999 Jun)** 26 (6) 1102-6.
Journal code: 8306785. ISSN: 0736-6205.
AB The yeast three-hybrid system presents a valuable tool for detecting and analyzing RNA-protein interactions in vivo. A major drawback of the use of such a transcriptional reporter-based assay in a **library screen** is the frequent occurrence of false-positive results due to bait RNA-independent activation of the **reporter gene**. To minimize the isolation of false positives in three-hybrid **library screens**, we incorporated a rapid and simple procedure based on differential sensitivity to 5-fluoro-orotic acid. The technique effectively eliminates bait RNA-independent false positives and thus greatly enhances the efficiency of the yeast three-hybrid system.

L4 ANSWER 7 OF 43 MEDLINE on STN
TI Targeted gene delivery to mammalian cells by filamentous bacteriophage.
AU Poul M A; Marks J D
PY 1999
SO Journal of molecular biology, **(1999 Apr 30)** 288 (2) 203-11.
Journal code: 2985088R. ISSN: 0022-2836.
AB We report that prokaryotic viruses can be re-engineered to infect eukaryotic cells resulting in expression of a **reporter gene** inserted into the bacteriophage genome. Phage capable of binding mammalian cells expressing the growth factor receptor ErbB2 and undergoing receptor-mediated endocytosis were isolated by selection of a phage antibody **library** on breast tumor cells and recovery of infectious phage from within the cell. As determined by immunofluorescence, F5 phage were efficiently endocytosed into 100 % of ErbB2 expressing SKBR3 cells. To achieve **reporter gene** expression, F5 phage were engineered to package the green fluorescent protein (GFP) **reporter gene** driven by the CMV promoter. These phage when applied to cells underwent ErbB2-mediated endocytosis leading to GFP expression. GFP expression occurred only in cells overexpressing ErbB2, was dose-dependent reaching, 4 % of cells after 60 hours and was detected with phage titers as low as 2.0 x 10(7) cfu/ml (500 phage/cell). The results demonstrate that bacterial viruses displaying the appropriate antibody can bind to mammalian receptors and utilize the endocytic pathway to infect eukaryotic cells, resulting in expression of a **reporter gene** inserted into the viral genome. This represents a novel method to discover targeting molecules capable of delivering a gene intracellularly into the correct trafficking pathway for gene expression by directly screening phage antibodies. This should significantly facilitate the identification of appropriate targets and targeting molecules for gene therapy or other applications where delivery into the cytosol is required. This approach can be adapted to directly select, rather than **screen**, phage antibodies for targeted gene expression. The results also demonstrate the potential of phage antibodies as an in vitro or in vivo targeted gene delivery vehicle.

L4 ANSWER 8 OF 43 MEDLINE on STN
TI Identification of RNAs that bind to a specific protein using the yeast three-hybrid system.
AU Sengupta D J; Wickens M; Fields S
PY 1999
SO RNA (New York, N.Y.), **(1999 Apr)** 5 (4) 596-601.
Journal code: 9509184. ISSN: 1355-8382.
AB We have adapted the yeast three-hybrid system to identify RNA ligands for an RNA-binding protein. In this assay system, a protein-RNA interaction is detected by the reconstitution of a transcriptional activator using two hybrid proteins and a hybrid RNA. The RNA molecule is tethered to the promoter of a **reporter gene** by binding to a hybrid protein consisting of the bacteriophage MS2 coat protein fused to the DNA-binding protein LexA; the RNA-binding domain to be analyzed is fused to the transcriptional activation domain of the yeast Gal4 protein; and the bifunctional RNA consists of binding sites for the coat protein and for the other RNA-binding domain. We built an RNA **library** such that short fragments of genomic DNA from yeast were transcribed in yeast together with binding sites for the coat protein. We screened this hybrid RNA **library** for RNAs that bound to the yeast Snp1 protein, a homolog of the human U1-70K protein. The **screen** yielded as the strongest positive the fragment of U1 RNA that contains loop I, which is known to bind to Snp1 in U1 snRNP. We also identified four other RNA ligands that produced weaker three-hybrid signals, suggesting lower affinities for Snp1 as compared to U1 RNA. In addition, this search also yielded a set of RNA sequences that can activate transcription on their own when bound to a promoter through a protein interaction.

L4 ANSWER 9 OF 43 MEDLINE on STN
TI Partial characterization of the Streptomyces lividans xlnB promoter and its use for expression of a thermostable xylanase from Thermotoga maritima.
AU Chen C C; Westpheling J
PY 1998
SO Applied and environmental microbiology, **(1998 Nov)** 64 (11) 4217-25.
Journal code: 7605801. ISSN: 0099-2240.
AB Xylanase activity assays were used to **screen** a Streptomyces coelicolor genomic **library** in Escherichia coli, and a xylanase gene that is 99% identical to the xylanase B gene (xlnB) of S. lividans (GenBank accession number M64552) was identified. The promoter region of this gene was identified by using a transcriptional fusion between the upstream region of the S. coelicolor xlnB gene and the xylE **reporter gene**. Transcription from the xlnB promoter was found to be induced by xylan and repressed by glucose. A single apparent transcription start site was identified by both primer extension analysis and in vitro run off transcription assays. Analysis of deletions of the promoter identified a region required for glucose repression. By using the transcriptional and protein localization signals of the Streptomyces xlnB gene, an in-frame translational fusion between the end of the xlnB signal sequence and the ATG of the Thermotoga maritima xynA gene was constructed. The xynA gene encodes a thermostable xylanase that has been demonstrated to be useful in the bleaching of Kraft pulp. The xlnB-xynA gene fusion was expressed in Streptomyces, and the activity of the protein produced was thermostable and was localized to the supernatant fraction of harvested cells.

L4 ANSWER 10 OF 43 MEDLINE on STN
TI Development of a yeast trihybrid **screen** using stable yeast strains and regulated protein expression.
AU Fuller K J; Morse M A; White J H; Dowell S J; Sims M J

PY 1998
SO BioTechniques, **(1998 Jul)** 25 (1) 85-8, 90-2.
Journal code: 8306785. ISSN: 0736-6205.
AB We describe a yeast trihybrid system that facilitates rapid screening of cDNA **libraries**. Novel yeast vectors were developed that direct integration of cDNA encoding the bait and third protein component into the yeast chromosome. A recombinant yeast strain is thus generated (screening strain) and is available for **library** transformation. Transformation with the **library** DNA is a single, efficient transformation event, allowing the cDNA **library** to be represented in one step. Recovery of the **library** plasmid from the yeast is also simplified, since it is the only episomal plasmid. Assay of trihybrid interaction and identification of positive clones is facilitated by regulating expression of the third protein component using the yeast MET3 promoter, which is repressed in the presence of exogenous methionine. Trihybrid interactions are detected only on media lacking methionine. This trihybrid system uses the standard E. coli LacZ and yeast HIS3 **reporter genes** and is compatible with most available Gal4 activation domain cDNA **libraries**. We describe the successful application of this yeast trihybrid system to the study of phosphoprotein interactions involved in T-cell signaling.

=> d l4 11-20 ti au py so ab

L4 ANSWER 11 OF 43 MEDLINE on STN
TI Grb10 identified as a potential regulator of growth hormone (GH) signaling by cloning of GH receptor target proteins.
AU Moutoussamy S; Renaudie F; Lago F; Kelly P A; Finidori J
PY 1998
SO Journal of biological chemistry, **(1998 Jun 26)** 273 (26) 15906-12.
Journal code: 2985121R. ISSN: 0021-9258.
AB The cloning of receptor targets procedure, used so far to identify proteins associated with tyrosine kinase receptors was modified to clone SH2 proteins able to bind to the growth hormone receptor (GHR). The cytoplasmic region of GHR, a member of the cytokine receptor superfamily does not contain tyrosine kinase activity. It was thus phosphorylated in bacteria by the Elk tyrosine kinase and radiolabeled to **screen** a mouse expression **library**. With this probe, we identified Shc and the p85 subunit of phosphatidylinositol 3-kinase as direct targets of the receptor. The other proteins identified, Csk, Shb, Grb4, and Grb10 are new potential transducers for cytokine receptors. We show in Huh-7 hepatoma cells that Grb10 and GHR associate under GH stimulation. Co-transfections in 293 cells further show that Grb10 interacts with both the GHR and Jak2. Functional tests demonstrate that Grb10 inhibits transcription of two **reporter genes** containing, respectively, the serum response element of c-fos and the GH response element 2 of the Spi2.1 gene, whereas it has no effect on a **reporter gene** containing only Stat5 binding elements. Our results suggest that Grb10 is a new target for a member of the cytokine receptor family that down-regulates some GH signaling pathways downstream of Jak2 and independently of Stat5.

L4 ANSWER 12 OF 43 MEDLINE on STN
TI A novel protein distinguishes between quiescent and activated forms of the type I transforming growth factor beta receptor.
AU Charng M J; Zhang D; Kinnunen P; Schneider M D
PY 1998
SO Journal of biological chemistry, **(1998 Apr 17)** 273 (16) 9365-8.
Journal code: 2985121R. ISSN: 0021-9258.
AB Transforming growth factor beta (TGFbeta) signal transduction is mediated

by two receptor Ser/Thr kinases acting in series, type II TGFbeta receptor (TbetaR-II) phosphorylating type I TGFbeta receptor (TbetaR-I). Because the failure of interaction cloning, thus far, to identify bona fide TbetaR-I substrates might reasonably have been due to the use of inactive TbetaR-I as bait, we sought to identify molecules that interact specifically with active TbetaR-I, employing the triple mutation L193A,P194A,T204D in a yeast two-hybrid system. The Leu-Pro substitutions prevent interaction with FK506-binding protein 12 (FKBP12), whose putative function in TGFbeta signaling we have previously disproved; the charge substitution at Thr204 constitutively activates TbetaR-I. Unlike previous **screens** using wild-type TbetaR-I, where FKBP12 predominated, none of the resulting colonies encoded FKBP12. A novel protein was identified, TbetaR-I-associated protein-1 (TRAP-1), that interacts in yeast specifically with mutationally activated TbetaR-I, but not wild-type TbetaR-I, TbetaR-II, or irrelevant proteins. In mammalian cells, TRAP-1 was co-precipitated only by mutationally activated TbetaR-I and ligand-activated TbetaR-I, but not wild-type TbetaR-I in the absence of TGFbeta. The partial TRAP-1 protein that specifically binds these mutationally and ligand-activated forms of TbetaR-I can inhibit signaling by the native receptor after stimulation with TGFbeta or by the constitutively activated receptor mutation, as measured by a TGFbeta-dependent **reporter gene**. Thus, TRAP-1 can distinguish activated forms of the receptor from wild-type receptor in the absence of TGFbeta and may potentially have a functional role in TGFbeta signaling.

L4 ANSWER 13 OF 43 MEDLINE on STN
TI Genetic recombination as a reporter for screening steroid receptor agonists and antagonists.
AU Dias J M; Go N F; Hart C P; Mattheakis L C
PY 1998
SO Analytical biochemistry, **(1998 Apr 10)** 258 (1) 96-102.
Journal code: 0370535. ISSN: 0003-2697.
AB Reporter cell lines are often used for high throughput screening of chemical **libraries** to identify new receptor ligands. Here we show how Cre recombinase can be used in mammalian cells to **screen** for steroid receptor ligands. A translational fusion of Cre recombinase and the ligand binding domain of the human glucocorticoid receptor was transfected into mammalian cells with a loxP/luciferase **reporter gene**. The recombinase function of the fusion is dependent on ligand binding to the receptor, and Cre-mediated recombination results in constitutive expression of luciferase from the **reporter gene**. A stable transfected clone was isolated and used to characterize the kinetics, ligand specificity, and dose response to various receptor ligands. The Cre fusion system, unlike a transcriptional reporter using the mouse mammary tumor virus promoter, can detect binding of the receptor antagonist RU486. We also studied the Cre reporter in a sensitive, miniaturized, assay format using an 864-well plate and show that as few as 560 cells per assay well was sufficient to measure a dose response to ligand.

L4 ANSWER 14 OF 43 MEDLINE on STN
TI Organization, 5'-flanking sequence and promoter activity of the rat GPC1 gene.
AU Asundi V K; Keister B F; Carey D J
PY 1998
SO Gene, **(1998 Jan 12)** 206 (2) 255-61.
Journal code: 7706761. ISSN: 0378-1119.
AB Glypicans are a member of a family of glycosylphosphatidylinositol anchored heparan sulfate proteoglycans that are expressed in cell and development specific patterns. Rat GPC1 cDNA probes were used to

screen rat genomic **libraries**. Three overlapping genomic clones that contained the entire rat GPC1 gene were isolated. The rat GPC1 gene is approximately 15kb in length and consists of eight exons interrupted by introns of varying lengths. Two of the introns are quite short, with lengths of 41 and 43 base pairs. Each exon-intron splice junction exhibited the consensus splice site sequence. Exon 1 encodes the putative signal peptide and the serine residue of the first putative heparan sulfate attachment site. The last exon encodes the cluster of three potential COOH-terminal heparan sulfate attachment sites, the putative GPI anchor and polypeptide cleavage site, and the 3'-untranslated region including the polyadenylation signal. One of the genomic clones extended approximately 2.8 kb 5' of the exon 1 coding sequence, and is thus likely to contain sequences that regulate GPC1 gene expression. Sequence analysis of the 5'-flanking sequence revealed a lack of consensus TATA and CAAT boxes. A search for potential transcription factor binding sites revealed a number of such motifs, including Sp1 (GC box), NF-kappaB, and MyoD (E-box). This region of the rat GPC1 gene shows significant sequence homology to the 5'-flanking region of the human GPC3 gene. Functional promoter activity of the rat GPC1 sequence was demonstrated by its ability to drive the expression of a luciferase **reporter gene** in several cell types.

L4 ANSWER 15 OF 43 MEDLINE on STN
TI Selection of peptides that functionally replace a zinc finger in the Sp1 transcription factor by using a yeast combinatorial **library**.
AU Cheng X; Boyer J L; Juliano R L
PY 1997
SO Proceedings of the National Academy of Sciences of the United States of America, **(1997 Dec 9)** 94 (25) 14120-5.
Journal code: 7505876. ISSN: 0027-8424.
AB We have developed a strategy for the identification of peptides able to functionally replace a zinc finger domain in a transcription factor. This strategy could have important ramifications for basic research on gene regulation and for the development of therapeutic agents. In this study in yeast, we expressed chimeric proteins that included a random peptide combinatorial **library** in association with two zinc finger domains and a transactivating domain. The **library** was screened for chimeric proteins capable of activating transcription from a target sequence in the upstream regulatory regions of selectable or **reporter genes**. In a **screen** of approximately 1.5 x 10(7) transformants we identified 30 chimeric proteins that exhibited transcriptional activation, some of which were able to discriminate between wild-type and mutant DNA targets. Chimeric **library** proteins expressed as glutathione S-transferase fusions bound to double-stranded oligonucleotides containing the target sequence, suggesting that the chimeras bind directly to DNA. Surprisingly, none of the peptides identified resembled a zinc finger or other well-known transcription factor DNA binding domain.

L4 ANSWER 16 OF 43 MEDLINE on STN
TI The basic helix-loop-helix-zipper transcription factor USF1 regulates expression of the surfactant protein-A gene.
AU Gao E; Wang Y; Alcorn J L; Mendelson C R
PY 1997
SO Journal of biological chemistry, **(1997 Sep 12)** 272 (37) 23398-406.
Journal code: 2985121R. ISSN: 0021-9258.
AB Expression of the rabbit pulmonary surfactant protein A (SP-A) gene is lung-specific, occurs primarily in type II cells, and is developmentally regulated. We previously identified two E-box-like enhancers, termed the distal binding element (DBE) and proximal binding element (PBE), in the 5'-flanking region of the rabbit SP-A gene. In the present study, the PBE

was used to **screen** a rabbit fetal lung cDNA expression **library**; a cDNA insert was isolated which is highly similar in sequence to human upstream stimulatory factor 1 (hUSF1). By use of reverse transcription polymerase chain reaction, two isoforms of rabbit USF1 (rUSF1) mRNAs were identified in fetal rabbit lung and other tissues. The levels of rUSF1 mRNAs reach a peak in fetal rabbit lung at 23 days gestation, in concert with the time of initiation of SP-A gene transcription. Binding complexes of nuclear proteins obtained from fetal rabbit lung tissue and isolated type II cells with the DBE and PBE were supershifted by the addition of anti-rUSF1 IgG. Binding activity was enriched in type II cells compared with lung fibroblasts. Overexpression of rUSF1s in A549 adenocarcinoma cells positively regulated SP-A promoter activity of cotransfected **reporter gene** constructs. It is suggested that rUSF1s, which bind to two E-box elements in the SP-A gene 5'-flanking region, may serve a key role in the regulation of SP-A gene expression in pulmonary type II cells.

L4 ANSWER 17 OF 43 MEDLINE on STN
TI Interaction of ATF6 and serum response factor.
AU Zhu C; Johansen F E; Prywes R
PY 1997
SO Molecular and cellular biology, **(1997 Sep)** 17 (9) 4957-66.
Journal code: 8109087. ISSN: 0270-7306.
AB Serum response factor (SRF) is a transcription factor which binds to the serum response element (SRE) in the c-fos promoter. It is required for regulated expression of the c-fos gene as well as other immediate-early genes and some tissue-specific genes. To better understand the regulation of SRF, we used a yeast interaction assay to **screen** a human HeLa cell cDNA **library** for SRF-interacting proteins. ATF6, a basic-leucine zipper protein, was isolated by binding to SRF and in particular to its transcriptional activation domain. The binding of ATF6 to SRF was also detected in vitro. An ATF6-VP16 chimera activated expression of an SRE **reporter gene** in HeLa cells, suggesting that ATF6 can interact with endogenous SRF. More strikingly, an antisense ATF6 construct reduced serum induction of a c-fos **reporter gene**, suggesting that ATF6 is involved in activation of transcription by SRF. ATF6 was previously partially cloned as a member of the ATF family. The complete cDNA of ATF6 was isolated, and its expression pattern was described.

L4 ANSWER 18 OF 43 MEDLINE on STN
TI Reconstitution of the NF-kappa B system in Saccharomyces cerevisiae for isolation of effectors by phenotype modulation.
AU Epinat J C; Whiteside S T; Rice N R; Israel A
PY 1997
SO Yeast (Chichester, England), **(1997 Jun 15)** 13 (7) 599-612.
Journal code: 8607637. ISSN: 0749-503X.
AB NF-kappa B is a ubiquitous transcription factor that contributes to the induction of many genes playing a central role in immune and inflammatory responses. The NF-kappa B proteins are subject to multiple regulatory influences including post-translational modifications such as phosphorylation and proteolytic processing. A very important component of this regulation is the control of their subcellular localization: cytoplasmic retention of NF-kappa B is achieved through interaction with I kappa B molecules. In response to extracellular signals, these molecules undergo degradation, NF-kappa B translocates to the nucleus and activates its target genes. To investigate novel proteins involved in this dynamic response, we have reconstituted the NF-kappa B/I kappa Beta system in the yeast Saccharomyces cerevisiae. We have successively introduced p65, the main transcriptional activator of the NF-kappa B family, which leads to the activation of two **reporter genes** controlled by kappa B sites, and the I kappa B alpha inhibitory protein, which abolishes

this activation. By transforming such a yeast strain with a cDNA **library** we have performed a genetic **screen** for cDNAs encoding proteins capable of either dissociating the p65/I kappa B alpha complex or directly transactivating the expression of the **reporter genes**. The efficiency of our **screen** was demonstrated by the isolation of a cDNA encoding the p105 precursor of the p50 subunit of NF-kappa B. We also used this system to test stimuli known to activate signalling pathways in yeast, in order to investigate whether the related mammalian cascades might be involved in NF-kappa B activation. We showed that yeast endogenous kinase cascades activated by pheromone, hypo- or hyperosmotic shock cannot modulate NF-kappa B activity in our system, and that the p38 human MAP kinase does not act directly on the p65/I kappa B alpha complex.

L4 ANSWER 19 OF 43 MEDLINE on STN
TI A three-hybrid system for detecting small ligand-protein receptor interactions.
AU Licitra E J; Liu J O
PY 1996
SO Proceedings of the National Academy of Sciences of the United States of America, **(1996 Nov 12)** 93 (23) 12817-21.
Journal code: 7505876. ISSN: 0027-8424.
AB Small ligand-receptor interactions underlie many fundamental processes in biology and form the basis for pharmacological intervention of human diseases in medicine. We report herein a genetic system, named the yeast three-hybrid system, for detecting ligand-receptor interactions in vivo. This system is adapted from the yeast two-hybrid system with which a third synthetic hybrid ligand is combined. The feasibility of this system was demonstrated using as the hybrid ligand a heterodimer of covalently linked dexamethasone and FK506. Yeast expressing fusion proteins of the hormone binding domain of the rat glucocorticoid receptor fused to the LexA DNA-binding domain and FKBP12 fused to a transcriptional activation domain activated **reporter genes** when plated on medium containing the dexamethasone-FK506 heterodimer. The **reporter gene** activation is completely abrogated in a competitive manner by the presence of excess FK506. Using this system, we screened a Jurkat cDNA **library** fused to the transcriptional activation domain in yeast expressing the hormone binding domain of rat glucocorticoid receptor-LexA DNA binding domain fusion protein in the presence of dexamethasone-FK506 heterodimer. We isolated overlapping clones of human FKBP12. These results demonstrate that the three-hybrid system can be used to discover receptors for small ligands and to **screen** for new ligands to known receptors.

L4 ANSWER 20 OF 43 MEDLINE on STN
TI Human phenol sulfotransferase gene contains two alternative promoters: Structure and expression of the gene.
AU Bernier F; Soucy P; Luu-The V
PY 1996
SO DNA and cell biology, **(1996 May)** 15 (5) 367-75.
Journal code: 9004522. ISSN: 1044-5498.
AB Phenol sulfotransferases catalyze the transfer of a sulfonate moiety from 3'-phosphoadenosyl 5'-phosphosulfate to a phenolic group of lipophylic substrates to generate soluble sulfate esters. Using a phenol sulfotransferase cDNA as probe to **screen** a human leukocyte genomic DNA **library** constructed in lambda EMBL3, we obtained a clone containing a complete gene sequence. Comparison of the gene sequence with that of the corresponding cDNAs, namely phenol-sulfating phenol sulfotransferase (P-PST) or thermostable sulfotransferase (TS-PST), and human aryl sulfotransferase 1 and 2 (HAST1 and HAST2) indicates that the gene possesses eight short exons separated by seven introns included in approximately 5 kb. HAST2 has a different 5' untranslated sequence,

and thus is encoded by a different mRNA species. While the nucleotide sequence corresponding to the 5' noncoding region of P-PST (TS-PST and HAST1) is included in the exon I, the 5' untranslated sequence of HAST2 is located in the beginning of exon IIa. The remaining sequence in exon II that is identical to both P-PST and HAST2 was termed exon IIb. Exons III to VIII, which cover the coding region and the 3' untranslated region, are almost identical in all types of PST or AST cDNAs. These results suggest that the phenol sulfotransferase gene possesses two alternate promoters that drive the expression of the two different mRNA species in a tissue-specific manner. Transfection of chloramphenicol acetyl transferase (CAT) **reporter gene** vectors containing the 5'-flanking sequence upstream from exon I and exon II, respectively, in transformed human embryonal kidney (293) cells indicate that both sequences possess promoter activity with higher activity for promoter 1. RNA blot analysis indicates that human phenol sulfotransferase gene is expressed in kidney, liver, lung, leukocyte, colon, small intestine, and spleen.

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L2	0	S	L1 AND GROWTH SIGNAL
L3	0	S	L1 AND ASSAY
L4	4930	S	AMINOTRIAZOLE OR CANAVANINE
L5	0	S	L4 AND GROWTH SIGNAL
L6	169	S	L4 AND ASSAY
L7	36	S	L6 AND GENE
L8	5	S	L7 AND MEDIUM
L9	4	DUP REM	L8 (1 DUPLICATE REMOVED)
L10	4	S	L9 AND PY <2002

L10 ANSWER 1 OF 4 MEDLINE on STN
TI Mitotic sectored colonies: evidence of heteroduplex DNA formation during direct repeat recombination.
AU Ronne H; Rothstein R
SO Proceedings of the National Academy of Sciences of the United States of America, **(1988 Apr)** 85 (8) 2696-700.
Journal code: 7505876. ISSN: 0027-8424.
PY 1988
AB In yeast meiosis, ascosporal colonies are sometimes sectored for a marker--i.e., half the colony has one allele and half has the other. This is interpreted as replicative resolution of heteroduplex DNA (hDNA) formed as a recombination intermediate. We have looked for similar evidence of hDNA formation during mitotic recombination between two repeated sequences on the same chromosome. The two repeats, an ochre suppressor and a wild-type tRNA **gene**, are separated by plasmid DNA and the URA3 marker. Recombination between the repeats excises the URA3 **gene** and one copy of the repeat, leaving either the wild-type tRNA or the suppressor on the chromosome. A red/white color **assay** is used to distinguish between the two. We find that some colonies that have lost the URA3 **gene** are sectored for the suppressor. This suggests that hDNA is formed across the anticodon during the recombination event and then resolved by replication. The disruption of either of two **genes** involved in recombination and repair, RAD1 and RAD52, does not significantly alter the frequency of sectored colony formation during plasmid excision.

L10 ANSWER 2 OF 4 MEDLINE on STN
TI Genomic DNA-mediated **gene** transfer for argininosuccinate synthetase.
AU Su T S; O'Brien W E; Beaudet A L
SO Somatic cell and molecular genetics, **(1984 Nov)** 10 (6) 601-6.
Journal code: 8403568. ISSN: 0740-7750.
PY 1984
AB **Canavanine**-resistant (Canr) human cells overproduce argininosuccinate synthetase without the occurrence of **gene** amplification. Using calcium phosphate precipitation, genomic DNA from Canr human cells was used to carry out **gene** transfer into Chinese hamster cells, which do not express argininosuccinate synthetase activity. Growth in tissue culture **medium** with citrulline substituted for arginine was adequate to select enzyme-positive colonies. Six independent isolates were selected for detailed analysis by enzyme **assay**, Southern blotting, Northern blotting, and S1 nuclease analysis, the last of which distinguishes human and hamster mRNA. Five isolates were transferrants containing the human structural **gene** and synthesizing human enzyme. One isolate represented a cell line synthesizing Chinese hamster enzyme. The data document **gene** transfer of DNA fragments at least 80 kb in length, the low level of spontaneous activation of the argininosuccinate synthetase locus in Chinese hamster cells, the feasibility of this expression and selection system for DNA-mediated **gene** transfer, and a method for distinguishing the human and hamster **gene** products at an RNA level.

L10 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
TI Compositions and methods for the quantification of sterol biosynthetic flux
IN Hopper, Anita K.; Martin, Nancy C.; Benko, Ann
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
PY 2000
2004

AB A novel **assay** for the detection of substances agonistic or antagonistic to the mevalonate pathway and sterol and cholesterol synthesis are disclosed. This **assay** incorporates colorimetric, growth, and immunol. methods for high throughput screening of compds. The method comprises (a) providing (i) a test compound, (ii) a growth **media** formulated to allow scoring of nonsense suppression in yeast and (iii) modified yeast cells expressing reduced cytosolic levels of Mod5p or its homolog as compared to the wild type yeast cells, and wherein the modified yeast cells comprise a **gene** with a nonsense mutation and a suppressor tRNA **gene** coding for a tRNA modified with isopentenyl adenosine by Mod5 or its homolog; (b) exposing a portion of the modified yeast cells to the test compound and the growth **media** to create a treated portion and an untreated portion; and (c) measuring for growth of the treated portion.

L10 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
TI UV-induced mutagenesis of human p53: analysis using a double-selection method in yeast
AU Moshinsky, Deborah J.; Wogan, Gerald N.
SO Environmental and Molecular Mutagenesis (**2000**), 35(1), 31-38
CODEN: EMMUEG; ISSN: 0893-6692
PY **2000**
AB Comparison of the mutation patterns of p53 in human tumors with those of selectable **genes** in model systems in a powerful approach to identify potential etiol. factors for specific tumor types. Recently, use of a yeast **assay** was validated to permit direct determination of the mutation spectrum induced in human p53 by carcinogens that would reduce uncertainties inherent in comparing spectra induced in different target **genes**. Modifications in the **assay** are designed to facilitate screening for mutants and to permit intracellular exposure of the **gene** instead of in vitro treatment. This was accomplished by introducing growth-based selection for transactivation-deficient p53 mutants into yeast already possessing red/white colony color selection. This improved model system was able to detect cells harboring p53 mutations among cells with wild-type p53 at a frequency of 10-4 or less. Addnl., UV light was used to verify that the majority of mutagenized cells with the appropriate phenotype on selective **medium** contained mutations in p53, not elsewhere in the genome. Sequence anal. of UV-induced mutations revealed that the nature of the mutations was similar to those obtained in previous studies of this mutagen. This system will prove useful in the determination of the ability of environmental agents to mutate the human p53 **gene**, and thus may contribute to hazard identification.